Vaccine Cuisine

Joseph Tart

In a field on the west coast of Africa, banana trees stand some 25 feet tall. The trees' huge frondlike leaves shadow clusters of ripening fruit, peels green in the midday sun. Each tree supports up to 150 bananas, enough to feed a crowd of people.

In coming years, trees like these may provide more than sweet nutrition. Bananas could become the world's first edible vaccine. "It may sound unusual," says Charles Arntzen, a plant biologist and president of Cornell University's Boyce Thompson Institute for Plant Research. "But you have to realize that many people in the developing world don't have access to the equipment needed for modern vaccines. They *do* have access to agriculture. So plants could be a way to deliver vaccines."

According to the World Health Organization (WHO), more than 2 million children—most in the developing world—die each year from diseases that can be prevented with vaccines. Bacterial diarrheas, which sicken adults but can dehydrate and kill children, are a major problem. Researchers at Boyce Thompson hope to develop oral plant vaccines to prevent deadly diarrhea caused by *Escherichia coli* and *Vibrio cholera* bacteria.

Like injected vaccines, the edible variety would not cause disease, but would train the body's immune system to recognize and attack a disease bacteria or virus. Unlike injected vaccines, however, the edible ones would be inexpensive and easy to distribute.

"Current recombinant vaccines are expensive because they need fermentation and protein purification," explains Hugh Mason, a molecular biologist at Boyce Thompson. "If you can instead produce the immunogenic protein in an edible plant tissue, you can lower the cost. I can envision growing large plots of plants in a way that makes [vaccine] production very cheap."

Gene shuttle. *Agrobacterium* carry genes for disease proteins into fruit cells. (*Source:* Hugh S. Mason)

Scientists at Boyce Thompson hope to use a straightforward bacterial vector approach to engineer life-saving bananas. First, they will splice the gene for an immune system-stimulating disease protein into *Agrobacterium*. When exposed to banana cells, the *Agrobacterium* will shuttle the gene inside. Researchers will then grow the cells into mature plants bearing fruit that, when eaten, confer immunity to the particular disease.

These researchers have already used this technique to engineer potatoes with a modified *E. coli* protein from a bacterial strain that normally causes severe diarrhea. Eating the raw potatoes, mice developed antibodies to the *E. coli* toxin. Theoretically, humans who eat the potatoes should develop similar immunity. "We've demonstrated feasibility," Arntzen says. "Now, we want to demonstrate efficacy." Arntzen and collaborators at a national vaccine testing center hope to begin human clinical trials with the *E. coli*-carrying potatoes this year.

"It's very exciting work," says Joseph St. Geme, a pediatrician at Washington University School of Medicine in St. Louis, Missouri. "There is increasing evidence that the oral immunization route is going to be effective. And it's as likely to be effective with a banana as with any other oral method of introducing an antigen."

At Scripps Research Institute in La Jolla, California, plant biologist Mich Hein is also studying oral vaccine delivery. Hein is growing alfalfa spiked with a modified cholera toxin, which should act as a vaccine when fed to mice. He hopes to study the highly immunogenic cholera toxin as a prelude to other diseases, particularly those that affect cattle.

"If we can find out what makes the modified cholera toxin an active oral vaccine in mice, we might be able to translate that into other diseases and other animals," Hein explains. "For example, it could be cheaper for the agriculture industry to deliver vaccines this way. And once we have addressed the safety and efficacy issues in nonhuman populations, we could move on to people."

John Clements, a microbiologist at Tulane University who collaborates with Arntzen, agrees. "The good thing about cattle or sheep is that you can control their daily diet," Clements adds. "You can clone an antigen into their alfalfa or hay feedstock, and the animals will consume large, consistent quantities. It's very practical."

Ultimately, the researchers envision officials in the developing world distributing fresh vaccine-carrying fruit to villagers. Medicinal bananas might sport a different colored peel—thanks to an engineered pigmentation gene—that distinguishes it from normal fruit. "With simple distribution," Arntzen says, "we might be able to do a lot of good."

The Perfect Vaccine

In 1992, WHO and other international organizations launched the Children's Vaccine Initiative, a cooperative effort to promote vaccine research for the developing world. That year, during a trip to Thailand, Arntzen was standing near a floating market when he noticed a mother running her finger along the top of a banana, lifting a taste of the fruit to her infant's lips. The scene stayed with him. "It just seemed so obvious to me," says Arntzen, then at Texas A&M University. "Bananas would be the perfect food for an oral vaccine." The fruit is cheap, abundant, and often fed to children, he notes. "And most importantly, it is eaten uncooked, so the vaccine would not be destroyed by heating."

At the time, researchers elsewhere had engineered a hepatitis B virus (HBV) vaccine into yeast cells. "I wondered whether it might be possible to produce a similar recombinant vaccine in plants," Arntzen says. It was an unusual idea. "Some people dismissed the idea as totally unworkable," Arntzen says. "They gave us a look like we were naive plant molecular biologists who didn't understand immunology."

Still, Arntzen decided to try. His lab first chose tobacco, a well-understood plant, to test the plant vaccine idea. They spliced a gene encoding an HBV surface protein into *Agrobacterium*. Invading the tobacco cells, the *Agrobacterium* directed the cells to produce the HBV antigen. The researchers regenerated the cells into tobacco plants.

Inspecting electron micrographs of tobacco tissue extracts, Arntzen was thrilled at what he saw: tiny, viruslike particles of HBV. "It was incredible. To be honest, I almost expected the plants to degrade the antigen. Plants usually do that to nonuseful proteins. And here we come along, putting in a protein of a human pathogen . . . it was very exciting that it worked."

Arntzen and colleagues next crushed the tobacco plants' leaves, extracted the HBV antigen, and injected it into mice. Sure enough, the mice developed antibodies to the antigen.

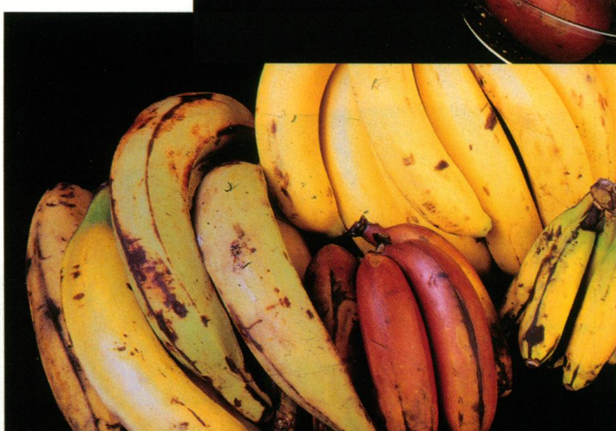
Now that the lab knew the technology worked, they turned to a well-known food crop—the potato. Using their modified HBV antigen, the researchers added a DNA sequence that causes proteins to be expressed in potato tubers. They used the same process as before and achieved the same result: antibodies surfaced.

Although injecting plant-produced vaccines in mice is a good way to test the concept, researchers really want animals to gain immunity by eating a vaccine in food. So far, mice that eat HBV-tainted potatoes have not responded with significant antibody production.

"We just don't know yet if we can get [the response] with HBV through an oral route," Arntzen says. On the other hand, the researchers have documented antibody response in mice feeding on potatoes laced with the *E. coli* toxin. Why the difference? It appears that the *E. coli* toxin, a potent immune system stimulator, is better than HBV at alerting immune cells secreted into the digestive system. This makes sense, given that the *E. coli* protein causes a digestive disorder.

The researchers are also pursuing potato studies with Norwalk virus, which is responsible for about half the outbreaks of gastroenteritis in the United States. Mary Estes, a molecular virologist at Baylor College of Medicine in Houston, is a key collaborator in the Norwalk work.

"We had already produced the Norwalk virus capsid protein in another system [insect cells] and knew it self-assembled into particles which were immunogenic when given orally to animals," Estes said. "So I was optimistic that we could make similar particles in plants, and this was a great idea." At press time, the group's first report showing that Norwalk virus expressed in potatoes is immunogenic had been submitted for publication.



Veggie vectors. Children may soon have another reason for eating their fruits and vegetables: to get vaccinated.

For all its potential, the potato remains a somewhat impractical edible vaccine crop. "For one thing, you really need something that can be eaten raw," says Gregory May, a plant biologist at Boyce Thompson. "If you cook the potato, its engineered proteins are going to denature and lose their function. And who eats raw potatoes?"

Enter the banana, a food almost always eaten raw. "In the developing world," May says, "people eat bananas all the time. They make banana flour and banana beer. And they feed bananas to young children."

Step by step, May and colleagues are engineering a vaccine-carrying banana. Last spring, the group reported its first milestone: they successfully engineered a marker gene into the fruit. They will soon attempt engineering vaccine antigens as well.

The banana research involves collaboration with a research lab in Irapuato, Mexico, which has field facilities to grow the tropical fruit. "We would like to establish a collaboration with Mexico's Ministry of Health," Arntzen says. "They have a good infrastructure for public education about health issues." In that country, he notes, diarrheal disease is the number one killer of children under five.

Technical Hurdles

Three years ago, no one had ever tried to engineer a vaccine-carrying plant. "We're starting with near–zero information on inserting foreign pharmacological proteins in plants," Arntzen says. "So we do a lot of empirical groundwork." Much of that work involves boosting gene expression. Often, an engineered disease gene produces a protein in plant tissue, but at levels too low to generate an immune response if the plant were eaten.

"Gene expression is a continuing challenge," notes Hein. One way researchers hope to maximize expression is to learn how some naturally occurring genes become highly expressed in the first place. "We're trying to identify proteins present in abundance in the banana," explains May. "Then we can backtrack to look for the regulatory elements that determine the proteins' expression." Researchers would then attach the DNA sequences that drive high gene expression to their disease antigen gene. Using this technique, the group increased

E. coli protein expression in potatoes tenfold, Mason says.

Once researchers get enough of a disease protein into a plant, the next challenge is to make sure an animal's immune system responds to that protein. One problem is oral tolerance. "Many antigens that you eat in food do not give an immune response," Mason says. "That's because the body perceives the food antigen as food, and the immune response is essentially repressed."

To alert the immune system, the researchers may try to pair vaccine disease proteins with very strong oral immunogens, like the cholera toxin or Norwalk virus. These adjuvants may help stimulate an immune response.

Meanwhile, a lot of questions remain. "They range all the way from kitchen science, like what happens to the protein when you crush alfalfa, dry it, and make it into food, to long-term immunization issues," Hein says.

The next goal for plant vaccine engineers is to demonstrate that protective immunity is gained by eating an engineered plant. So far, the experiments with mice have shown only that they generate antibodies when presented with a disease antigen. These mice have never actually encountered natural *E. coli* or HBV diseases, because animals don't get these human diseases.

The only way to truly test whether a plant vaccine confers protective immunity is to deliver that plant to a human, who is then exposed to the disease under investigation. "This is the acid test," says Mason. "In some cases, an antigen preparation may stimulate immunoglobulins that neutralize the antigen in the test tube and yet provide little or no protection against the disease. We are planning to begin some human studies with the potatoes, initially only to assess safety and immunogenicity in humans, but later to assess protection."

Clinical trials should answer a host of questions, including whether normal plant proteins interfere with disease antigen delivery or how well various immunizing proteins act when taken orally. Clements, for one, is confident the plant vaccine technology will prevail. "It's going to take some time to develop," he says. "But there sure is a lot of potential."

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Suggested Reading

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